MANAGING PATHOGENS OF GREENHOUSE GERANIUMS WITH BIOLOGICAL FUNGICIDES

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A thesis submitted in partial fulfillment of requirements for graduation *with research distinction* in the undergraduate College of Food, Agriculture, and Environmental Sciences of The Ohio State University

The Ohio State University

2011

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ABSTRACT

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The amount of pesticides used annually by the agriculture industry is nearly a billion pounds, worth over US $31 billion. According to the USDA, biological fungicides are beneficial fungi and bacteria that attack and control plant pathogens. The advantages of biological fungicides are reduced risks to the applicator, reduced number of applications, a shortened re-entry interval, and a potential to control both soilborne and foliar pathogens.

Botrytis cinerea causes blight, a very common and destructive disease of greenhouse crops that is estimated to cause the largest economic loss of all ornamental diseases. Cease is a biological fungicide with the active ingredient *Bacillus subtilis* Strain QST 713, a ubiquitous naturally occurring bacterium that controls many plant fungal pathogens. The common practice recommended by plant pathologists of using different modes of action was incorporated into an experiment for controlling *B. cinerea* with a biological fungicide. Veranda O has the active ingredient of polyoxin D zinc salt, a byproduct of the soil bacterium *Streptomyces cacaoi* var. *asoensis*. The mode of action is to interfere with the fungal cell wall production by inhibiting chitin synthase. In this experiment, both biological fungicides were used alone and in alternating combinations, each starting with a different mode of action. Fungicides were sprayed once per week for four weeks inoculating with a spore suspension four hours later. Plants were bagged to maintain high humidity. Neither Cease nor Veranda O used alone gave significant disease control. However, using alternating combinations of the two biological fungicides on a weekly basis significantly reduced the sporulation of *B. cinerea*. The Cease/Veranda O combination gave the highest level of disease control when compared to the other treatments. The combination starting with Cease and then Veranda O on a weekly basis provided a comparable level of control to a fungicide commonly used by the greenhouse industry to manage botrytis gray mold. This data set shows that rotations between different types of biological fungicides may be suitable alternatives to conventional fungicides.

Pythium spp. cause root rot on a variety of crops and is a serious disease of greenhouse geraniums. Rootshield is a biological fungicide currently available with the active ingredient *Trichoderma harzianum* Rifai strain KRL-AG2. *Trichoderma* spp. are fungi that are naturally present in many soils and habitats. They are known to colonize plant roots readily and grow on the roots as they develop. In addition to colonizing roots, *Trichoderma* spp. have been known to attack and parasitize other fungi through many different mechanisms. There has been limited work with Rootshield as a biological fungicide and its ability to control pythium root rot of geraniums. Rootshield was tested to determine its effectiveness in controlling root rot caused by two different pythium species in a controlled environment. This data set shows that using Rootshield to control two different pythium species can work in certain instances and depends on the environment and the inoculum load.

ACKNOWLEDGMENTS

A word of appreciation is extended to my research advisor Dr. Dennis Lewandowski, my thesis and academic advisor Dr. Thomas Mitchell, and my professor and friend Dr. Landon Rhodes.

This research was funded in part by a grant from the Ohio State University College of Food, Agriculture, and Environmental Sciences honors department. Gratitude is also expressed to Ecke Ranch for providing the endless supply of geraniums used in these experiments.

Thanks also to Jim Uphaus (the Statistician); Mike Kelly (the Lab Supervisor); Justin Morse (the greenhouse sprayer); Veronique Beckrum and Chris Woltjen for advice and courage; Caleb Schlegel for great friendship; and Ryon and Nancy Hoffstetter for encouragement and support at home.

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INTRODUCTION

Fungicides have been used for decades to control common fungal diseases in the nursery and greenhouse industries. The types of fungicides used have dramatically changed and developed over time. The first use of a "fungicide" occurred in the 17th century, when the brining of grain with salt water followed by liming took place to control bunt. The technique was developed following the observation that wheat seed removed from the ocean was free of blunt. In 1807, Prevost discovered bunt and smuts of cereals could be controlled by wetting wheat kernels with a copper sulfate solution (Klittich, 2008). In 1885, Millardet noticed that grapes sprayed with a mixture of copper sulfate and lime, to deter pilferers, retained their leaves, whereas those left unsprayed lost their leaves (Klittich, 2008). Millardet concluded that a mixture of copper sulfate and hydrated lime (Bordeaux mixture) could effectively control downy mildew of grape, and the first fungicide was produced (Morton and Stuab, 2008). In 1913, the first organic fungicide was introduced in Germany, an organomercurial seed treatment (Klittich, 2008). Up till the 1940s, chemical disease control relied heavily on inorganic chemicals prepared by the user. Between 1940 and 1970 a new era of fungicides became mainstream. The major components of this era were the dithiocarbamates followed by the phthalimides. These chemistries represented a major improvement over the inorganic fungicides. They were more active, less phytotoxic, and easier to prepare for the user. During the 1960s and the 1970s, there was a rapid expansion of research and development in relation to the fungicide market (Morton and Stuab, 2008). This decade produced the most widely used protectant fungicides, mancozeb and chlorothalonil. It also gave rise to the first broad-spectrum foliar systemic fungicide (thiabendazole) and systemic seed treatment (carboxin). These new chemistries arose due to the new technology *in vivo* screens instead of *in vitro* screens. In 1969, the first synthetic seed treatment was introduced, carbocin, to control surface- borne smuts and bunts and also smut

infections within the seed (Klittich, 2008). The first broader spectrum fungicide that provided systemic and curative activity was introduced by DuPont called benomyl, was introduced in 1970 (Klittich, 2008). After the 1970s, more modern fungicides were introduced. These included the triazoles, the largest class of fungicides, and the strobilurins which are now the second largest chemistry group (Morton and Stuab, 2008). The strobilurins were introduced in 1996, with the introduction of kresoxim-methy from BASF (Klittich, 2008). Many other forms of strobilurins have been introduced in the years since. Recently due to environmental and regulatory pressures, there has been an effort to develop fungicides with a more indirect mode of action. These fungicides are not toxic to the isolated fungus, and are more environmentally friendly. Several have modes of action that stimulate the host plant's natural defenses, such as acibenzolar-S-methyl (Klittich, 2008) have been released. These compounds provide a great deal of benefit to growers since they do not selectively pressure fungal growth and evolution, and are considered more durable than conventional fungicides.

Disease and pest control are a major production expense. The total amount of pesticides used by the agriculture industry in 2007 was 5.1 million pounds (United States, 2011). The amount of conventional pesticides used was 857 million pounds. Fungicides accounted for 70 million pounds of pesticide usage. In 2007, the world pesticide expenditure totaled more than US $39.4 billion (United States, 2011). In many cases, multiple fungicides and applications must be made to a crop to control diseases caused by a variety of different pathogens. Approaches are being sought to reduce the impact of conventional fungicides through a reduction in the number of applications, the increased use of reduced-risk materials, and using alternatives such as biorational and/or biological fungicides. According to the USDA, biological fungicides are beneficial fungi and bacteria that attack and control plant pathogens and disease.

These products are often based on small molecules that induce a defense response in the plant against pathogens. A number of biological fungicides based upon various fungal and bacterial species (e.g. *Trichoderma* spp. and *Bacillus* spp.) have been commercialized (Daughtrey and Benson, 2005). Some organisms used as biological fungicides have shown the ability to induce plant defenses, which in turn can fend off the advancing plant pathogen (Horst et al., 2005). *Trichoderma* has been shown to protect seedlings from damping off (root infection by pathogens) and enhance plant growth (Harman et al., 1989). Bicontrol agents also provide unique protection compared to synthetic fungicides. These organisms continue to grow and proliferate; therefore they can colonize and protect newly formed plant parts to which they were not initially applied, providing an expanded window of protection during the growing season (Harman, 1990).

Growers are interested in management practices that reduce pesticide use. However, they are also skeptical of new technologies until thoroughly vetted. To be adopted, any new approach must be cost effective and perform as reliably as the currently available chemicals. More research is needed with biological or bio-based pest management tools to determine their application to reducing the amounts of conventional pesticides used in the nursery and greenhouse industries. For a biological fungicide (sometimes referred to as a biocontrol agent) to be adopted it must:

1. Reduce the risks
2. Save growers money and reduce input costs
3. Reduce the number of applications
4. Reduce the re-entry interval (REI).

Biological fungicides reduce the risks associated with the use of conventional pesticides by reducing the potential of exposure to the pesticide applicator and by reducing off-target drift. Some of these fungicides, such as Rootshield, can be applied through chemigation (the application of a chemical or pesticide through an irrigation system) where there is less risk for the person operating the spray equipment and less worker exposure as the applications are made to the soil. They save growers money and reduce input costs because they reduce the number of applications made. The application to the roots reduces the likelihood that it will be exposed to other fungicides that are applied to the foliage (Horst et al., 2005). Also, using a biocontrol agent that controls both foliar and soilborne pathogens could reduce the input costs associated with multiple fungicides. Many biological fungicides remain active in the soil or on the plant surface reducing the amount of sprays or drenches needed. Finally, biological fungicides reduce the re-entry interval (REI), with most having a re-entry period around 4 hours. This is less than most conventional pesticides. This lower REI level allows workers to re-enter the treated area sooner to resume work.

Many biological fungicides are currently available on the market for the use by greenhouse and nursery industries. Biological control is becoming a critical component of plant disease management for the production of specialty crops. Pesticide registrations are being lost every year due to their safety and environmental impacts. Some chemical fungicides that are registered for use are unavailable to growers because of pressures and concerns from the general public. Three biocontrol fungicides are Rootshield, Cease, and Veranda O. Rootshield contains a fungus species that is currently found in the soil, water, and air. Cease and Veranda O contain bacteria and secondary metabolites of bacteria. These active ingredients make the chemicals safer for humans because they attack only pathogenic fungi and bacteria of plants.

While there are many benefits to using a biological fungicide over a conventional fungicide, there is little research done with biological fungicides on ornamentals to convince growers that they should use biocontrol methods over conventional. The project presented here was done to determine if biological fungicides can indeed control foliar and soilborne fungal diseases of greenhouse geraniums as effectively as a conventional fungicide commonly used by the industry.

Two important diseases in the greenhouse industry are botrytis gray mold and pythium root rot of geraniums. One of the most commonly found and a destructive disease of greenhouse crops is gray mold. It is estimated that it causes more economic loss in the greenhouse industry than any other disease. It occurs mostly during cool, damp, and cloudy weather and is a major problem during shipment of most types of horticultural commodities ("Botrytis blight or gray mold of ornamental plants," 1997). The fungus invades and damages many plant parts including: flowers, pedicels, stems, leaves, buds, fruits, bulbs, corms, tubers, and roots. However, botrytis blight mainly attacks the tender, injured, and senescing tissues. Actively growing tissues besides the flower rarely become infected. There are over 50 species of *Botrytis* that cause blight, however *Botrytis cinerea* has the largest host range of them all. Most ornamental plants are susceptible to one or more species of *Botrytis* ("Botrytis blight or gray mold of ornamental plants," 1997).

All species of greenhouse plants are susceptible to one or more soilborne fungus capable of causing damping off of the seedlings or crown rot of mature plants. This disease is the most serious to the grower because it is hard to recognize and control. Entire flats are often lost to damping off. If the plants survive, they are often week and nonproductive. Pythium root rot is a primary problem of root rot in seedlings and basal rot of cuttings for propagation; however, they

can be associated with root rot of established plants ("Damping-off and root rots of house plants and garden flowers," 1988). This rot is favored by cool, wet, poorly drained soils and overwatering. Pythium typically infects the younger feeding roots and then advances into the entire root system and causes a wet odorless rot. Roots often take a light brown to black discoloration after rot starts to occur. Typically the outer portion of the root (cortex) becomes soft and slimy and it can be separated from the inner core (stele) ("Damping-off and root rots of house plants and garden flowers," 1988). If the disease is severe enough, it can move into the stem and ultimately turn it slimy and black. Pythium can survive for several years in the soil on plant residue or as thick walled resistant spores ("Damping-off and root rots of house plants and garden flowers," 1988). These experiments were done to test whether biological fungicides can control these two pathogens of greenhouse geraniums as effectively as a conventional fungicide commonly used by the industry.

OBJECTIVE 1, PHASE 1

The first objective was to determine if biological fungicides were effective in controlling *Botrytis cinerea* of greenhouse geraniums. Two experiments were done to determine the control of a biological fungicide in comparison with the control provided by a readily available conventional fungicide.

Fungicides

The biological fungicide used in this experiment was Cease which is an aqueous suspension biofungicide with the active ingredient of *Bacillus subtilis* strain QST713. This strain of Bacillus is a naturally occurring widespread bacterium commonly found in the soil, water, and the air. It's mode of action is that it controls the growth of certain harmful bacteria and fungi by competing for nutrients and growth sites on plants, and by directly colonizing and attaching to fungal pathogens (United States, 2000). The conventional fungicide used in this experiment was Daconil Ultrex with the active ingredient chlorothalonil. Chlorothalonil, in chemical family nitriles, is a broad spectrum contact or protectant fungicide with a long residual activity. It helps to prevent infection by fungi on the plant when applied as a protective barrier on the plant surface. It works as a multi-site inhibitor affecting various enzymes used by the fungus to respire and other metabolic processes by inactivating amino acids, proteins, and enzymes when it combines with amino and thiol groups. It inhibits spore germination, and is toxic to fungal cell membranes.

MATERIALS AND METHODS

Plant Material

Plant material was donated by Ecke Ranch in Encinitas, California. Sixty four zonal geraniums (*Pelagronium* x *hortorum* 'Patriot White') were used for this experiment. The plants arrived 2 weeks apart and were transplanted into 6 inch pots. The geranium plugs arrived in February 4, 2010, and were four months old when the experiment took place. Plants were watered on a regular basis and fertilized with Miracle Grow once a week until the experiment was started.

Inoculation and Treatments

This experiment was done in a greenhouse environment. Plants were placed on a greenhouse bench in randomized complete block design. Both experiments were identical and contained seven treatments (Table 1) with six replicates per treatment. The first experiment was sprayed on June 2nd, 2010. The second experiment was sprayed and inoculated two days later on June 4th, 2010. Two rates (Table 1) of Cease were used on inoculated plants and un-inoculated plants to determine if the biological fungicide had an effect on plant growth. Fungicides were sprayed using a pump sprayer outside until runoff. Inoculations were made four hours after plants were sprayed. *Botrytis cinerea* cultures were grown on potato dextrose agar for three and a half weeks under a fluorescent light before each inoculation. Spore concentrations were determined using a hemacytometer and diluted to 2.9 x 10^7 conidia/fl. oz. (Webster et al., 2004). The spores were then placed into a 1/10 dilution suspension with water and applied with a Delta all purpose hand sprayer bottle applying on average 30 mL of spore concentration per plant.

After spraying, plants were placed in a hanging basket with the wire bent and a plastic bag with air to maintain moisture and high relative humidity.

Ratings and Statistical Analysis

Plant ratings were taken four days after inoculation (June 8th and 11th, 2010) and again two weeks later (June 22nd and 25th, 2010). Ratings were taken of the total number of leaves, the number of infected leaves, the number of sporulating leaves, and the overall disease severity. The percent of infected leaves was calculated manually by dividing the number of infected leaves by the total number of leaves. The percent of sporulating leaves was calculated in the same manner. The overall disease severity was determined on a scale of 1 to 10, where 1= healthy, 2 to 8 ascending degrees of blighting, and 10=dead (Webster et al., 2004). Data was analyzed using SAS and an analysis of variance was conducted to determine the significance of treatment.

Results

Disease pressure was slight to moderate and all plants were infected to some degree. The results showed that after one week none of the treatments were statistically different from the untreated inoculated control, however the mean for the inoculated Cease High rate was significantly lower than the untreated control. After the first week, the inoculated Cease High and Daconil treatments were statistically different from the inoculated untreated control at reducing the percent of sporulating leaves. Cease High and Daconil were not statistically different from the inoculated Cease Low treatment. After two weeks, the Cease High inoculated and the Daconil treatments were the only treatments statistically lower than the untreated inoculated control at the percent of infected leaves (Table 2).

OBJECTIVE 1, PHASE 2

In phase two of the first objective, an additional fungicide was incorporated into the spray schedule to alternate modes of action. Spraying was done using two biological fungicides alone and in alternative combinations in comparison with the conventional fungicide.

Fungicides

The same fungicides used in phase 1 were used in phase 2. However, another biological fungicide was incorporated into the spray schedule to alternate mode of action. The second biological fungicide used was Veranda O. This fungicide is from the chemical family polyoxins which are produced by a soil bacterium and are naturally found in soils in Japan. The bacteria that produce polyoxins are grown commercially and then the polyoxins are then purified in the form of Polyoxin D with salt to provide longer stability on the plant. The antibiotic Polyoxin D is a secondary metabolite of the soil bacterium *Streptomyces cacaoi var. asoensis* and functions as a cell wall inhibitor by inhibiting the chitin synthase enzyme. It also inhibits spore germination and mycelia growth on the plant surface ("Consideration of eligibility for registration of the new pesticide active ingredient Polyoxin D Zinc Salt-DECISION MEMORANDUM," n.d.).

MATERIALS AND METHODS

Plant Material

The geraniums used in this experiment were donated from Ecke Ranch. Thirty six zonal geraniums (*Pelagronium* x *hortorum* 'Patriot White') planted in February 10, 2010

were used for this experiment. Plants were grown in 5.5 square inch pots and watered and fertilized regularly. Plants were six months old when the experiment took place.

Inoculation and Treatments

This experiment was performed in a greenhouse. The plants were placed on a bench in a randomized complete block design. There were six treatments (Table 3) and six replicates per treatment. Fungicides were sprayed using a chamber sprayer made by Allen Machine Works (517-8351287, Pat # 5040733) that was modified using a one liter sparkling water bottle, a hose, spray nozzle, tubing, and fittings (Figure 1). The spray boom traveled at 0.045 meters per second and the psi was at 25. Three plants were placed on the spray chamber bench at a time and rotated 90 degrees between the two sprays (Figure 2). The rates (Table 3) of the fungicide were determined following the fungicide labels and the maximum rates were used to achieve the highest degree of control possible. A total of 200 mL of the fungicides were sprayed on each set of three plants. Sprays were made on a seven day interval for four weeks starting on October 29, 2010. Four hours after fungicide treatments were applied, the plants were sprayed with botrytis spores. *Botrytis cinerea* cultures were grown on potato dextrose agar for three and a half weeks under a fluorescent light before each inoculation. There were a total of four culture dates, one every week for four weeks. Spore concentrations were determined using a hemacytometer and diluted to 2.9×10^7 conidia/fl. oz. (Webster et al., 2004). The spores were then placed into a 1/10 dilution suspension with water. *Botrytis cinerea* spores were sprayed onto each plant two hours after fungicide application to allow time for drying. The spore suspension was applied using a Delta all purpose hand sprayer bottle (Figure 3) directly to the foliage until run off (Webster et al., 2004). After spraying, plants were placed in a hanging

basket with the wire bent and a plastic bag with air to maintain moisture and high relative humidity.

Ratings and Statistical Analysis

Botrytis ratings were taken ten days after the last inoculation (November 29, 2010). Ratings were taken by determining the total number of leaves, then determining the total number of leaves infected and the total number sporulating leaves. The overall disease severity was determined on a scale of 1 to 10, where 1= healthy, 2 to 8 ascending degrees of blighting, and 10=dead (Webster et al., 2004). Data was analyzed using SAS and a analysis of variance was conducted to determine the significance of treatment.

Results

All plants were infected with botrytis gray mold at the conclusion of the experiment (Figure 4). Veranda O used alone was not statistically different from the untreated control in all disease parameters measured (Table 4). The Cease, Daconil, and Cease/Veranda O treatments significantly reduced the disease severity relative to the untreated control. The mean disease rating for the Cease/Veranda O alternating treatment was the lowest (Figure 4), although not statistically different from the Daconil and Cease treatments (Table 4). The VerandaO/Cease, Daconil, and Cease/Veranda O treatments significantly reduced spourlation compared to the untreated control. Daconil also significantly reduced sporulation compared to Cease and Veranda O. The Cease and Cease/Veranda O treatments significantly reduced the percent infected leaves compared to the untreated control (Table 4 and Figure 4).

OBJECTIVE 2, PHASE 1

A biological fungicide was compared to a conventional fungicide on the effective control of two pythium root rot pathogens on greenhouse geraniums when used as a soil drench. The study was done to determine if biological fungicides work as well as conventional chemical control strategies.

Fungicides

Two fungicides were used in this experiment to determine the effectiveness of a biological fungicide compared to a conventional fungicide on the effective control of two pythium root rot pathogens on geraniums. Rootshield is a biological fungicide with the active ingredient *Trichoderma harzianum* Rifai strain KRL-AG2 (Figure 6). *Trichoderma* spp. are fungi that are present in nearly all soils and diverse habitats. They are favored by the presence of high levels of plant roots where they colonize the roots readily. Depending on the strain, once they come in contact with roots, they colonize the surface or cortex depending on the strain. In addition to colonizing the roots, *Trichoderma* spp. also attack, parasitize, and otherwise gain nutrition from other fungi enhancing plant and root growth. There are many mechanisms in which *Trichoderma* spp. attack other fungi. A recent list includes: mycoparasitism, antibiosis, competition for nutrients and space, tolerance to stress through enhanced root and plant development, solubilization and sequestration of inorganic development, solubilization and sequestration of inorganic nutrients, induced resistance, and inactivation of the pathogen's enzymes (Harman, 1976). The conventional fungicide used in this experiment Segway has the active ingredient cyazoafamid. Cyazoafamid, in the chemical family Cyanoimidazole, inhibits spore germination.

Pathogens and Symptoms

Pythium species are considered to be oomycetes, which are not true fungi, because they survive and grow best in wet soils. They produce sporangia which can turn into vesicles. These vesicles then give rise to 100 or more zoospores which are then released into the soil (Agrios, 2005). Zoospores use chemotaxis to find roots and have flagella that allow them to be mobile. Two pythium species, *Pythium ultimum* and *Pythium aphanidermatum*, were used in this experiment to determine the control methods of different fungicides in controlling this root rot disease. *P. ultimum* is a soilborne pathogen that causes damping off and root rot, and causes a dramatic economic loss in many different crops. This pathogen is widely distributed throughout the world and has a wide host range (Cheng, 2007). *P. aphanidermatum* is a pathogen known worldwide and it also has a vast host range (Parker, n.d.). It is a very aggressive species and causes damping off, root and stem rots, and blight of grasses and fruit. It is a major economic concern to most annuals, cucurbits, and grasses. Both pythium species favor warm temperatures making them an issue for most greenhouses, with *Pythium aphanidermatum* favoring higher temperatures. *P. aphanidermatum* has a minimum temperature of 50^{O}F and an optimum temperature of 95-100^{O}F. *P. ultimum* has a minimum growth temperature of 41^{O}F and a maximum temperature of 95^{O}F (Moorman et al., 2002). The optimum growth temperature for *P. ultimum* is between 77 and 86^{O}F. *P. aphnidermatum* readily produces zoospores in flooded soils, and is therefore well adapted at spreading in recirculating irrigation systems. The differences between the two species can be seen in their sporangia, or asexual spores. *P. aphanidermatum* have lobate (inflated) sporangia and produce zoospores. *P. ultimum* produces spherical sporangia that germinate directly (Moorman et al., 2002).

There are many symptoms of pythium root rot caused by these two pathogens. Pythium root rot can cause wilting, loss of vigor, stunting, chlorosis of the leaves, and even cause leaves to drop. These pathogens inhibit the root growth of the plant and turn the roots black, mushy, and eventually kill them. Most of the symptoms occur at the root tip first and quickly advance to the main roots. *P. aphanidermatum* also can cause infection in underground storage structures. *P. ultimum* cause the root tips to rot and turn brown. Taking a closer look microscopically at the root cells, round think walled spores called an oospore can be found (Parker).

MATERIALS AND METHODS

Plant Material

Plants were received from Ecke Ranch in Encinitas, California on March 18th, 2010 and transplanted on April 15th. Seventy two patriot white zonal geraniums were used for this experiment. Plants were transplanted into 4 inch pots, watered, and fertilized regularly with Miracle Grow.

Inoculation and Treatments

Plants were placed on a greenhouse bench in randomized complete block design with four blocks total. There were 19 treatments (Table 5) with four replicates per treatment. Fungicides were applied as a soil drench with 118 mL of Rootshield and 2 oz. of Segway being used for the respective treatments. The pre-treatments took place on April 16th, 2010. Inoculation and day of inoculation treatments took place on April 30th, 2010. Four hours after fungicide treatments, inoculum was delivered to the plants. *P. ultimum* and *P. aphanidermatum* were grown on V8 agar for three days. Four agar plugs were placed in the soil of the pots. After inoculation, plants were placed in weigh boats and watered to keep the plugs from drying out.

Ratings and Statistical Analysis

Root ratings were taken five and a half weeks after inoculation on June 9th, 2010. Root ratings were done using a scale of 1 to 5, where 1= 0% of roots infected, 2= 1-10%, 3= 11-25%, 4= 26-50%, and 5= 51-100% of the roots are infected (McGovern et al., 2001). Data was analyzed using SAS and an analysis of variance was conducted to determine the significance of treatment.

Results

Disease pressure was moderate. All plants had some degree of root rot. The high rate on the day of inoculation, high rate pretreatment and day of inoculation, high rate pre-treatment, and low rate pre-treatment and day of inoculation of Rootshield significantly reduced the root disease causes by *P. aphanidermatum* compared the untreated inoculated control (Figure 5). The conventional fungicide treatment Segway, the low pre-treatment/day of inoculation and high pre-treatment/day of inoculation treatments are not statistically different for *P. aphanidermatum*. The other treatments had no effect on reducing the root disease (Table 6). None of the Rootshield treatments had an effect on root disease with *P. ultimum* (Table 7and Figure 6).

OBJECTIVE 2, PHASE 2

The evaluation of a biological fungicide was compared to a conventional fungicide on the effective control of two pythium root rot pathogens on greenhouse geraniums when used as a soil drench. The experiment was done similar to objective one using a liquid suspension as inoculums.

Fungicides

The same fungicides, used in phase one, were used to in phase two of the objective.

Pathogens and Symptoms

The same two pythium species were used to inoculate phase two of the objective.

MATERIALS AND METHODS

Plant Material

Plants arrived on March 4th, 2010 and transplanted on March 17th. Sixty patriot white zonal geraniums were transplanted into 3 inch pots, watered, and fertilized regularly with Miracle Grow.

Inoculation and Treatments

Plants were placed on a greenhouse bench in randomized complete block design with four blocks total. There were 15 treatments (Table 8) in this experiment with four replicates per treatment. Fungicides were applied as a soil drench using 118 mL of Rootshield and 2 oz. of Segway respectively. Pre treatments took place on April 27th, 2010. Two weeks later (May 11th, 2010) the second treatment was made and four hours later the inoculum was applied. Pythium

species were grown for one week on V8 agar. Two plates were blended with 400 mL of sterile distilled water. 30 mL of this solution was applied to the plants four hours after treatments. After inoculation, plants were placed in weigh boats and watered to maintain wet soil conditions.

Ratings and Statistical Analysis

Disease severity ratings were taken three weeks after inoculation on June 3, 2010. Plants were rated on a scale of 1 to 5; 1= healthy, 2=minor wilting, 3=moderate wilting or chlorosis, 4=severe wilting or chlorosis, 5=plant death (Hausbeck et al., 2008). Data was analyzed using SAS and an analysis of variance was conducted to determine the significance of the treatment.

Results

Disease pressure was high and all plants had root rot symptoms. None of the treatments reduced the root disease when *P. aphanidermatum* was present (Table 9 and Figure 7). For *P. ultimum*, low rootshield pre-treatment and low rootshield pre-treatment/day of inoculation were not statictically different from the untreated uninoculated control (Figure 8). Although, they weren't statically different from the untreated inoculated *P. ultimum*, these two treatments did reduce the severity of the root rot (Table 10).

OBJECTIVE 2, PHASE 3

The evaluation of a biological fungicide compared to a conventional fungicide on the effective control of two pythium root rot pathogens on greenhouse geraniums when used as a soil drench in a controlled environment using a liquid suspension for inoculation.

Fungicides

Root shield was used as the biological fungicide in this experiment as in the two previous experiments. The conventional fungicide used was Subdue Maxx with the active ingredient mefenoxam. Mefenoxam is in the family Phenylamides which inhibit RNA synthesis.

Pathogens and Symptoms

The same two pythium species, used in phase one, were used to inoculate in phase three.

MATERIALS AND METHODS

Plant Material

Plants for this experiment were donated by Ecke Ranch. Thirty six patriot bright red zonal geraniums arrived on November 3rd, 2010 and were transplanted on November 10th into 4 inch pots. Plants were watered regularly and fertilized with Miracle Grow.

Inoculation and Treatments

This experiment was done in a controlled growth chamber model PGR15. On November 17, 2010 the growth chamber was set to 32^{O}C. Four metal halide lights (400 watts) and four high pressure sodium (HPS, 400 watts) lights were on for fourteen hours per day to provide an adequate light source. Lighting and temperature was adjusted to 26^{O}C and lights were reduced to

two metal halides and two HPS lights on November 23rd, 2010. The relative humidity was maintained throughout the experiment between 85 and 90%. Plants were placed in the growth chamber in four randomized complete design blocks (Figure 9). There were nine treatments (Table 11) in this experiment with four replicates per treatment. Fungicides were applied as a soil drench using 118 mL of Rootshield and 52.5 mL of Subdue Maxx respectively. Pre treatments took place on the day of transplanting November 10, 2010. One week later (November 17th) treatments were made with Rootshield and Subdue two days before inoculation. Two days later (November 19th), inoculum was applied using *Pythium* spp. grown on V8 agar for two days. Two plates were pureed with 400 mL of distilled water and decanted to remove agar particles. 5mL of the suspension was applied to each plant, pipetting 2.5 mL on each side of the geranium plug 1 cm deep (Figure 10) in the soil (Wick and Stone, 2007). After inoculation, plants were placed in the growth chamber in weigh boats and watered to maintain wet soil conditions.

Ratings

Shoot ratings were done twice during the course of the experiment. The first rating was taken three weeks after inoculation (December 9th, 2010) and the second rating was taken four and a half weeks after inoculation (December 22nd, 2010). Shoot ratings were done on a scale of one to five, 1- healthy, 2= slightly wilting, 3=moderate wilting, stunting, 4= severe wilting and stunting, and 5=dead (Wick et al., 2001). Shoot and root weights were taken seven weeks after inoculation (January 7th, 2011). The geranium roots were rinsed off to remove the dirt. Shoots were removed from the roots and weights were determined to one hundredth of a gram (Wick et al., 2007). Root ratings were also taken seven weeks after inoculation (Janurary 11th, 2011) on a scale of 1-4 with 1= white roots, 2= some visible rot rot, 3= some white roots, and 4= complete

root rot (Daughtery et al., 2001). Data was analyzed using SAS and an analysis of variance to determine the effect of treatment.

Results

Disease pressure was moderate to high and all infected plants had some degree of root rot. For controlling root rot of *Pythium ultimum*, High Rootshield used one week before and Subdue Maxx were not statistically different from the inoculated control in shoot and root weights (Figures 11, 12). However, High Rootshield one week before had a much higher mean for shoot weight than the Subdue Maxx and the inoculated control. It was also statistically higher than the untreated unioculated control in shoot weight. On *Pythium aphanidermatum*, Subdue Maxx used two days before was the only treatment that was statistically different from the untreated inoculated control in shoot and root weights (Figures 11, 12). Subdue Maxx was not statistically different from the untreated uninoculated control (Table 12).

DISCUSSION

In the first phase of the botrytis experiment, it was shown that the biological fungicide Cease used at high and low rates could reduce the percent of sporulating leaves comparably as a conventional fungicide such as Daconil. Also, it was shown that Cease used at high rate could reduce the percent of infected leaves relative to Daconil. This experiment showed that a biological fungicide had the potential to reduce the overall disease severity of botrytis gray mold.

Results from the first phase were used in the decision to alternate two different modes of action for biological fungicides in the second phase of this experiment. Alternating modes of action is a practice used by many plant pathologists to manage plant disease and reduce disease resistance. The results showed that neither Cease of Veranda O used alone provided significant control for botrytis gray mold of greenhouse geraniums. However, when the two biological fungicides were alternated on a seven day interval they provided a significant reduction in the sporulation of *Botrytis.* When the rotation was started with Cease first, the overall disease severity was greatly reduced (Figure 4). The interaction of the Veranda O and the Cease modes of action to control sporulation together at a higher rate than when used alone is unknown. It is also unknown as to why using Cease first and then Veranda O works better than starting with Veranda O first. The speculation is that the Cease mode of action, which inhibits germination by competing for nutrients and free water, is able to knock-down the fungus after sporulation occurs to reduce the overall disease severity.

The results of these two experiments shows that biological fungicides used in an alternating combination can provide growers a comparable level of control to conventional fungicides such as Daconil to control botrytis gray mold of greenhouse geraniums. Therefore, it

can be inferred that biological fungicides may be a suitable alternative to conventional fungicides in controlling botrytis gray mold in a greenhouse setting.

In the pythium root rot experiments, Rootshield at high rate worked to control the overall disease severity of *P. aphanidermatum* when the disease pressure was low and temperatures were cooler. However, when the temperatures were increased in the second and third phase of this experiment and the inoculums load was heavier the rootshield was no longer effective on *P. aphindermatum*. *P. aphanidermatum* is an aggressive pathogen and at high levels of inoculum and high temperatures it out competes the *Trichoderma harzianum* Rifai strain KRL-AG in the Rootshield. But when used at high rate one week before infection, it can be a comparable alternative to managing *P. aphanidermatum* if the disease pressures are low. For controlling *P. ultimum*, Rootshield was uneffective when the inoculums level was low. This could be caused by the lower temperatures in the greenhouse at this time creating a more conducive environment for *P. ultimum* to colonize the roots more readily than the *Trichoderma harzainum* before host resistance can be induced by the Trichoderma and out compete it for nutrients and space.

When disease pressure and temperature were high none of the treatments were effective at controlling *P. aphanidermatum*. *P. aphandermatum* is highly aggressive at high temperatures and at high inoculums loads it can colonize the roots and use nutrient sources faster than the Trichoderma can become established in the root system of the geraniums. Low rootshield pre-treatment and low rootshield pre-treatment/day of inoculation showed reduction in the severity of root rot although they were not statistically different from the untreated *P. ultimum*. However, this could be due to many factors. When the plants were placed in weigh boats, spores could have been splashed from plant to plant during watering. Also, at higher temperatures, *P.*

ultimum is not as aggressive and for one week the growth chamber was set to around 90^{O}F, which could have caused adverse effects on the pathogen.

At moderate disease pressures and controlled humidity and temperatures, none of the the rootshield treatments were effective at reducing the overall disease severity of *P. aphanidermatum*. In the growth chamber, temperatures and relative humidity were maintained at 90^{O}F for one week favoring a rapid development of *P. aphanidermatum* over the *Trichoderma harzainum* in Rootshield. When controlling *P. ultimum* at these conditions, Rootshield proved to be effective in reducing the disease severity. High Rootshield used one week before was not statistically different from the inoculated control which could be due to spore splashing in the growth chamber, the strain of *P. ultimum* could have been less pathogenic, or the cultures could have been too young to cause sufficient disease when inoculation occurred. While high Rootshield was not statistically different, it did have a much higher mean for shoot weight, indicating that the Rootshield might have an effect on the overall health of the plant. None of the *P. ultimum* Rootshield treatments were statistically different from the untreated unioculated control. The previous experiments showed that Rootshield did not have an effect on the overall plant health and size. However, in the growth chamber Rootshield could have had an effect on the individual size and vigor of the plant. This would explain why the Rootshield treatments were bigger than the untreated uninoculated control.

Biological fungicides are good alternatives to conventional fungicides because they have lower risks to the person spraying, they have a lower re-entry interval which would allow workers to re-enter the treated area sooner, and they could save the grower money by reducing the number of fungicide applications. This project has shown the biological fungicides can be

alternatives to conventional fungicides used in the greenhouse and nurseries when certain disease pressures and temperatures are met.

Table 1. Treatments for *Botrytis* Experiment Phase 1

Treatment	Pathogen	Rate
Untreated	Uninoculated	-
Untreated	Inoculated	-
Daconil	Inoculated	1.5 kg/1000 L
Cease	Inoculated	2 qts/100 gal
Cease	Inoculated	8 qts/100 gal
Cease	Uninoculated	2 qts/100 gal
Cease	Uninoculated	8 qts/100 gal

Table 2. Control of *Botrytis cinerea* with Biological Fungicides

Treatment	Pathogen	% Infected Leaves 1	% Infected Leaves 2	% Sporulating Leaves 1	% Sporulating Leaves 2	Disease Severity 1	Disease Severity 2
Untreated	Inoculated	11.25 a*	46.25 a	4.47 ab	41.28 a	3.08 a	3.17 a
Cease 8.0 qts	Uninoculated	8.77 a	44.85 ab	3.67 ab	40.13 a	2.25 b	3.17 a
Cease 2.0 qts	Uninoculated	10.74 a	42.19 ab	5.32 a	38.36 a	2.33 b	3.00 a
Untreated	Uninoculated	10.63 a	39.86 ab	4.98 a	35.43 a	2.33 b	3.17 a
Cease 2.0 qts	Inoculated	10.18 a	38.75 ab	2.55 bc	35.09 a	2.75 ab	3.00 a
Cease 8.0 qts	Inoculated	7.89 a	35.23 bc	1.31 c	31.88 ab	2.58 ab	2.83 ab
Daconil Ultrex 1.5 kg	Inoculated	7.69 a	27.26 c	0.94 c	23.83 b	2.25 b	2.58 b

*Numbers in a column with the same letter are not significantly different ($P<0.05$)

Table 3. Treatments for *Botrytis* Experiment Phase 2

Treatment	Pathogen	Rate
-	Inoculated	-
Cease	Inoculated	8 qts/100 gal
Veranda O	Inoculated	8 oz./100 gal
Daconil	Inoculated	1.5 kg/1000 L
Cease/ Veranda O*	Inoculated	8 qts/100 gal
		8 oz./100 gal
Veranda O/ Cease†	Inoculated	8 oz./100 gal
		8 qts/100 gal

* Veranda O was applied on weeks 1 and 3, Cease was applied on weeks 2 and 4

† Cease was applied on weeks 1 and 3, and Veranda O was applied on weeks 2 and 4

Table 4. Control of *Botrytis cinerea* with Biological Fungicides

Treatment	% Infected Leaves	% Leaves with Sporulation	Disease Severity
None	35.8 a≠	10.7 a	6.7 a
Veranda O 8.0 qts	27.7 ab	7.8 ab	5.3 ab
Veranda O 8.0 qts/Cease 8.0 qts*	30.7 ab	5.2 bc	5.3 ab
Cease 8.0 qts	24.7 b	6.7 ab	4.7 b
Daconil 1.5 kg	27.7 ab	2.0 c	4.7 b
Cease 8.0 qts/Veranda O 8.0 qts†	24.5 b	6.0 bc	4.3 b

* Veranda O was applied on weeks 1 and 3, Cease was applied on weeks 2 and 4

† Cease was applied on weeks 1 and 3, and Veranda O was applied on weeks 2 and 4

≠ Numbers in a column with the same letter are not significantly different ($P<0.05$)

Table 5. Treatments for *Pythium* Experiment Phase 1

Treatment	Pathogen	Rate
High Rootshield Pre-treatment*	*P. ultimum*	5.0 oz/100 gal
High Rootshield DOI†	*P. ultimum*	5.0 oz/100 gal
High Rootshield Pre & DOI	*P. ultimum*	5.0 oz/100 gal
Low Rootshield Pre-treatment	*P. ultimum*	3.0 oz/100 gal
Low Rootshield DOI	*P. ultimum*	3.0 oz/100 gal
Low Rootshield Pre & DOI	*P. ultimum*	3.0 oz/100 gal
Segway DOI	*P. ultimum*	3.0 oz/100 gal
High Rootshield Pre-treatment	*P. aphanidermatum*	5.0 oz/100 gal
High Rootshield DOI	*P. aphanidermatum*	5.0 oz/100 gal
High Rootshield Pre & DOI	*P. aphanidermatum*	5.0 oz/100 gal
Low Rootshield Pre-treatment	*P. aphanidermatum*	3.0 oz/100 gal
Low Rootshield DOI	*P. aphanidermatum*	3.0 oz/100 gal
Low Rootshield Pre & DOI	*P. aphanidermatum*	3.0 oz/100 gal
Segway	*P. aphanidermatum*	3.0 oz/100 gal
High Rootshield Pre-treatment	---	---
Low Rootshield Pre-treatment	---	---
Untreated	---	---
Untreated	*P. ultimum*	---
Untreated	*P. aphanidermatum*	---

* Pre-treatments were made on April 16, 2010

† Day of inoculation treatments (DOI) were made on April 30, 2010

Table 6. Control of Root Rot Caused by *P. aphanidermatum* using a Biological Fungicide

Treatment	**Pathogen**	**Root Rating**
Untreated	---	1.75 cd≠
Untreated	*P. aphanidermatum*	3.75 a
Rootshield Pre-Treatment* 3.0 oz	*P. aphanidermatum*	3.25 ab
Rootshield DOI† 3.0 oz	*P. aphanidermatum*	3.00 ab
Rootshield DOI 5.0 oz	*P. aphanidermatum*	2.75 b
Rootshield Pre-Treatment 5.0 oz	*P. aphanidermatum*	2.75 b
Rootshield Pre and DOI 3.0 oz	*P. aphanidermatum*	2.50 bc
Rootshield Pre and DOI 5.0 oz	*P. aphanidermatum*	2.50 bc
Rootshield Pre-treatment 3.0 oz	---	1.75 cd
Segway 3.0 oz	*P. aphanidermatum*	1.75 cd
Rootshield Pre-treatment 5.0 oz	---	1.50 d

* Pre-treatments were made on April 16, 2010

† Day of inoculation treatments (DOI) were made on April 30, 2010

≠Numbers in a column with the same letter are not significantly different (P<0.05)

Table 7. Control of Root Rot caused by *P. ultimum* with a Biological Fungicide

Treatment	**Pathogen**	**Root Rating**
Untreated	---	1.75 b≠
Untreated	*P. ultimum*	3.50 a
Rootshield Pre and DOI 3.0 oz	*P. ultimum*	3.75 a
Rootshield Pre-treatment* 5.0 oz	*P. ultimum*	3.50 a
Rootshield Pre & DOI 5.0 oz	*P. ultimum*	3.50 a
Rootshield Pre-treatment 3.0 oz	*P. ultimum*	3.50 a
Rootshield DOI† 3.0 oz	*P. ultimum*	3.50 a
Rootshield DOI 5.0 oz	*P. ultimum*	3.50 a
Segway DOI 3.0 oz	*P. ultimum*	1.75 b
Rootshield Pre-treatment 3.0 oz	---	1.75 b
Rootshield Pre-treatment 5.0 oz	---	1.50 b

* Pre-treatments were made on April 16, 2010

† Day of inoculation treatments (DOI) were made on April 30, 2010

≠Numbers in a column with the same letter are not significantly different (P<0.05)

Table 8. Treatments for *Pythium* Experiment Phase 2

Treatment	Pathogen	Rate
High Rootshield Pre-Treatment*	*P. ultimum*	5.0 oz/100 gal
High Rootshield Pre & DOI	*P. ultimum*	5.0 oz/100 gal
Low Rootshield Pre-treatment	*P. ultimum*	3.0 oz/100 gal
Low Rootshield Pre & DOI	*P. ultimum*	3.0 oz/100 gal
Segway DOI†	*P. ultimum*	3.0 oz/100 gal
Untreated	*P. ultimum*	---
High Rootshield Pre-Treatment	*P. aphanidermatum*	5.0 oz/100 gal
High Rootshield Pre & DOI	*P. aphanidermatum*	5.0 oz/100 gal
Low Rootshield Pre-treatment	*P. aphanidermatum*	3.0 oz/100 gal
Low Rootshield Pre & DOI	*P. aphanidermatum*	3.0 oz/100 gal
Segway DOI	*P. aphanidermatum*	3.0 oz/100 gal
Untreated	*P. aphanidermatum*	---
High Rootshield Pre-Treatment	---	5.0 oz/100 gal
Low Rootshield Pre-treatment	---	3.0 oz/100 gal
Untreated	---	---

* Pre-treatments were made on April 27, 2010

† Day of inoculation treatments (DOI) were made on May 11, 2010

Table 9. Control of Root Rot Caused by *P. aphanidermatum* with a Biological Fungicide

Treatment	**Pathogen**	**Shoot Rating**
Untreated	---	1.25 b≠
Untreated	*P. aphanidermatum*	4.00 a
Rootshield Pre & DOI 5.0 oz	*P. aphanidermatum*	4.25 a
Rootshield Pre-treatment* 5.0 oz	*P. aphanidermatum*	3.75 a
Segway DOI† 3.0 oz	*P. aphanidermatum*	3.75 a
Rootshield Pre-treatment 3.0 oz	*P. aphanidermatum*	3.50 a
Rootshield Pre & DOI 3.0 oz	*P. aphanidermatum*	3.25 a
Rootshield Pre-treatment 5.0 oz	---	1.25 b
Rootshield Pre-treatment 3.0 oz	---	1.00 b

* Pre-treatments were made on April 27, 2010

† Day of inoculation treatments (DOI) were made on May 11, 2010

≠Numbers in a column with the same letter are not significantly different ($P<0.05$)

Table 10. Control of Root Rot Caused By *P. ultimum* Using a Biological Fungicide

Treatment	**Pathogen**	**Shoot Rating**
Untreated	---	1.25 bc≠
Untreated	*P. ultimum*	2.50 a
Rootshield Pre-treatment* 5.0 oz	*P. ultimum*	3.25 a
Segway DOI † 3.0 oz	*P. ultimum*	3.00 a
Rootshield Pre & DOI 5.0 oz	*P. ultimum*	2.75 a
Rootshield Pre-treatment 3.0 oz	*P. ultimum*	2.25 ab
Rootshield Pre & DOI 3.0 oz	*P. ultimum*	2.25 ab
Rootshield Pre-treatment 5.0 oz	---	1.25 bc
Rootshield Pre-treatment 3.0 oz	---	1.00 c

* Pre-treatments were made on April 27, 2010

† Day of inoculation treatments (DOI) were made on May 11, 2010

≠Numbers in a column with the same letter are not significantly different ($P<0.05$)

Table 11. Control of *Pythium* Root Rot in Growth Chamber Using a Biological Fungicide

Treatment	Pathogen	Rate
Untreated	Uninoculated	---
Subdue Maxx	*P. aphanidermatum*	1.0 fl. oz. / 100 gal
Subdue Maxx	*P. ultimum*	1.0 fl. oz. / 100 gal
High Rootshield 2 Days†	*P. aphanidermatum*	5.0 oz/100gal
High Rootshield 2 Days	*P. ultimum*	5.0 oz/100 gal
High Rootshield 1 Week*	*P. aphanidermatum*	5.0 oz/100 gal
High Rootshield 1 Week	*P. ultimum*	5.0 oz/100 gal
Untreated	*P. aphanidermatum*	---
Untreated	*P. ultimum*	---

* Rootshield was applied one week before inoculation on 11/10/2010

†Rootshield and Subdue Maxx treatments were applied two days before inoculation on 11/17/2010

Table 12. Control of *Pythium* root rot using Rootshield as a Biological Fungicide

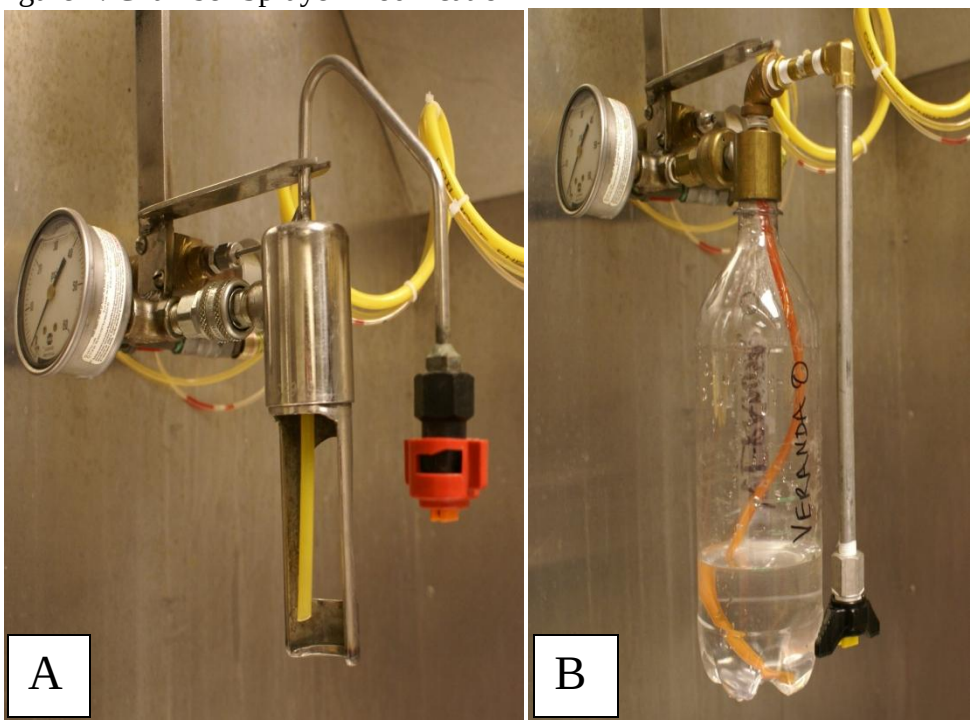
Treatment	Pathogen	Shoot Rating 2	Shoot Weight	Root Weight	Root Rating
Untreated	--	2.00 b	28.37 b≠	5.83 a	1.50 b
Untreated	*P. ultimum*	1.25 bc	33.75 ab	8.45 a	1.25 b
Untreated	*P. aphanidermatum*	5.00 a	0.99 c	0.00 b	4.00 a
RootShield*5.0 oz	*P. ultimum*	1.00 c	43.52 a	9.17 a	1.50 b
Subdue Maxx†1.0 fl. oz./	*P. ultimum*	1.50 bc	35.46 ab	8.45 a	1.00 b
Subdue Maxx1.0 fl. oz	*P. aphanidermatum*	1.5 bc	32.58 b	7.92 a	1.00 b
Rootshield†5.0 oz	*P. ultimum*	1.75 bc	30.09 b	9.17 a	1.25 b
High Rootshield*5.0 oz	*P. aphanidematum*	4.25 a	3.82 c	0.50 b	3.25 a
High Rootshield†5.0 oz	*P. aphanidermatum*	5.00 a	0.85 c	0.03 b	4.00 a

* Rootshield was applied one week before inoculation on November 10, 2010

†Rootshield and Subdue Maxx treatments were applied two days before inoculation on November 17, 2010

≠Numbers in a column with the same letter are not significantly different (P<0.05)

Figure 1. Chamber Sprayer Modification



A. Sprayer before modification. B. Sprayer after modifications were implemented

Figure 2. Spray Chamber

Plants were sprayed in the herbicide chamber three plants at a time and were sprayed twice with a total at 200 mL of fungicide.

Figure 3. Spraying *Botrytis* spores with hand sprayer

Botrytis cinerea spores were sprayed using a Delta all purpose hand spray bottle.

Figure 4. Representative zonal geranium plants inoculated with *Botrytis cinerea* for four consecutive weeks following treatment with biological or conventional fungicides.

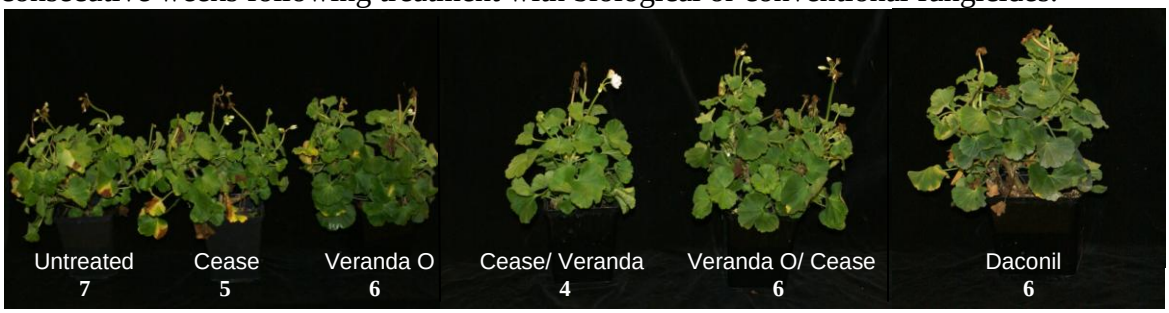
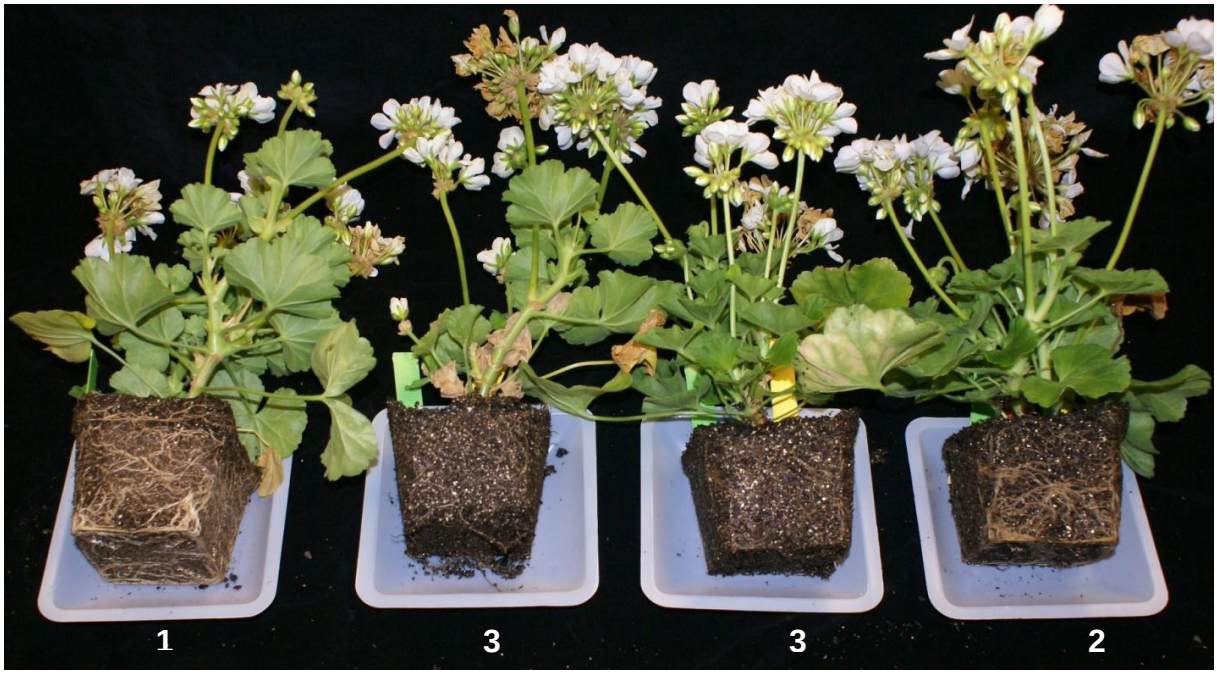
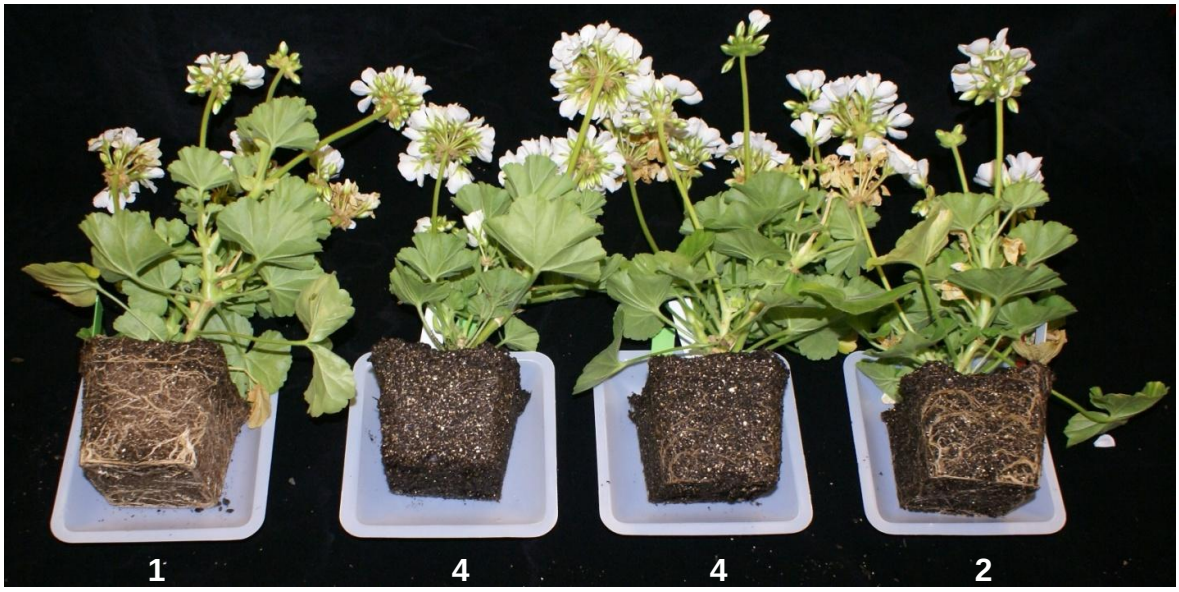


Figure 5. Representative root rots on zonal geranium plants inoculated with *Pythium aphanidermatum* following treatment with fungicides.



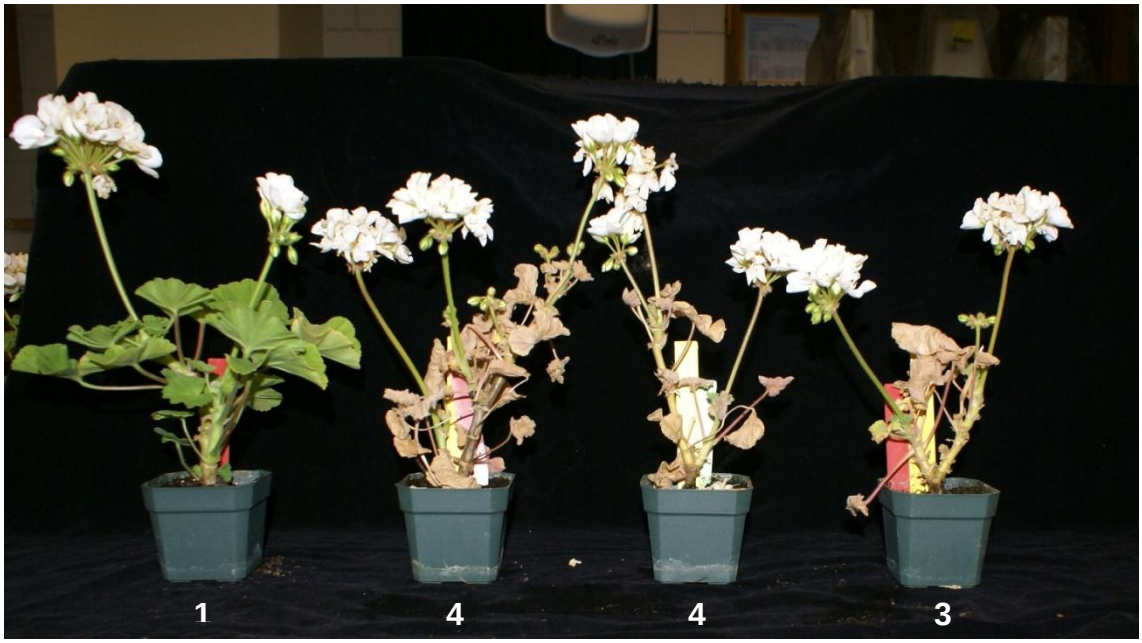
From left to right treatments and root ratings are: Untreated uninoculated, High Rootshield Pre-treatment and Day of Inoculation Treatment, Low Rootshield Pre-treatment and Day of Inoculation, Segway.

Figure 6. Representative root rots on zonal geranium plants inoculated with *Pythium ultimum* following treatment with fungicides.



From left to right the treatments and root ratings are: Untreated uninoculated, High Rootshield Pre-Treatment and Day of Inoculation, Low Rootshield Pre-treatment and Day of Inoculation, and Segway.

Figure 7. Representative plant health ratings on zonal geraniums plants inoculated with *Pythium aphanidermatum* following treatments with fungicides.



From left to right treatments and plant health ratings are: Untreated uninoculated, High Rootshield Pre-treatment and Day of Inoculation Treatment, Segway, Low Rootshield Pre-treatment and Day of Inoculation.

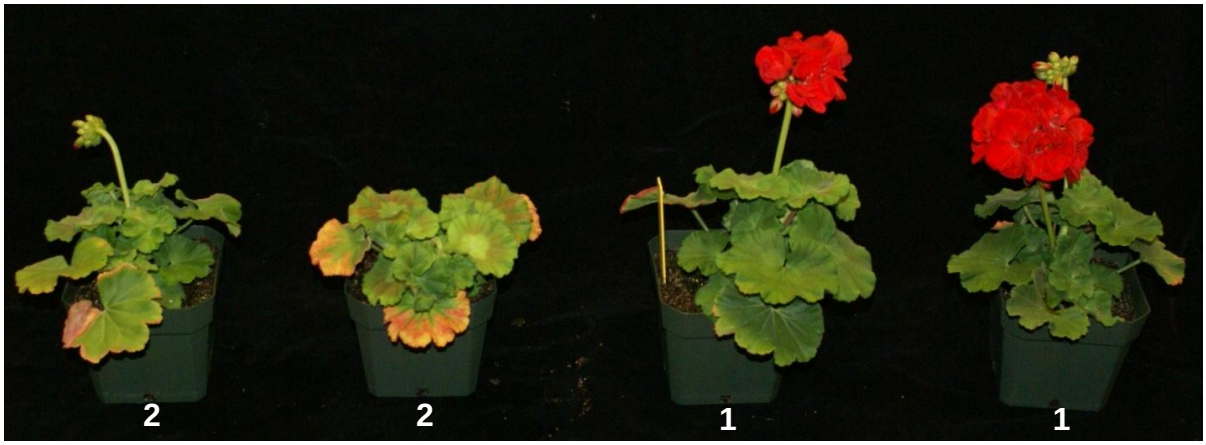
Figure 8. Representative plant health ratings on zonal geraniums inoculated with *Pythium ultimum* following treatments with fungicides.

From left to right treatments and plant health ratings are: Untreated uninoculated, High Rootshield Pre-treatment, Low Rootshield Pre-treatment and Day of Inoculation Treatment, Segway.

Figure 9. Plants were placed in a growth chamber in randomized complete block design.

Figure 10. Inoculation of *Pythium* spp.spore suspension.

Figure 11. Representative zonal geraniums on shoot ratings when using a biological fungicide to control *Pythium* spp.



From left to right *P. ultimum* treatments and shoot ratings: Subdue Max, High Rootshield 2 Days before Inoculation, High Rootshield 1 Week before Inoculation, Untreated Inoculated.

From left to right *P. aphanidermatum* treatments and shoot ratings: Subdue Maxx, High Rootshield 2 Days before Inoculation, High Rootshield 1 Week before Inoculation, Untreated Inoculated.

Figure 12. Representative zonal geraniums on root and shoot quality for *Pythium* root rot using a biological fungicide.

From left to right root and shoot quality of treatments inoculated with *P. ultimum*: Untreated uninoculated, Subdue Maxx 2 Days Before, High Rootshield 2 Days before Inoculation,High Rootshield 1 week before Inoculation , Untreated inoculated.

From left to right root and shoot quality of treatments inoculated with *P. aphanidermatum*: Untreated uninoculated, Subdue Maxx 2 Days before Inoculation, High Rootshield 2 Days before Inoculation, High Rootshield 1 Week for Inoculation, Untreated inoculated

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